Plant Physiol. (1993) 103: 665–666

***Plant Gene Register***

# Nucleotide Sequences of Two Peroxidase Genes from Tomato (*Lycopersicon esculentum*)

**Miguel A. Botella[1], Miguel A. Quesada, Paul M. Hasegawa, and Victoriano Valpuesta***

Departamento de Bioquimica y Biologia Molecular, (M.A.B., V.V.) and Departamento de Biologia Vegetal (M.A.Q.), Facultad de Ciencias, Universidad de Malaga, 29071 Malaga, Spain; and Center for Plant Environmental Stress Physiology, 1165 Horticulture Building, Purdue University, West Lafayette, Indiana 47907-1165 (P.M.H.)

Peroxidases (EC 1.11.1.7.) are heme enzymes that have been implicated in a large number of physiological processes in plants. Some of these processes include secondary cell wall biosynthesis (Abeles and Biles, 1991), wound healing (Espelie et al., 1986), polyphenol deposition after wounding (Lagrimini, 1991), auxin catabolism (Gaspar et al., 1989), and defense against pathogens (Ye et al., 1990). The presence of many peroxidases in higher plants makes it difficult to determine what is the role of each isozyme in vivo. According to the pI of the isozymes, they have been classified into three groups: cationic, moderately anionic, and anionic. Each group is proposed to have a different function in the cell (Lagrimini et al., 1987).

An oligonucleotide deduced from the conserved amino acid motif corresponding to the acid/base catalytic region (Phe-His-Asp-Cys-Phe-Val) was used to screen a tomato λ-gt10 cDNA library. Two positive clones, each containing a 1.2-kb insert, were isolated. The first clone (TPX1) contains an insert 1229 bp in length with an open reading frame of 984 nucleotides. It is predicted from the cDNA sequence that the protein is synthesized as a preprotein of $M_r$ 35,882 with a 22-amino acid N-terminal signal sequence. The predicted size of the mature peroxidase is 306 amino acids with a $M_r$ of 33,503. This mature protein has a theoretical pI of 6.16. The second cDNA clone (TPX2) encodes a preprotein of $M_r$ 35,505 (329 amino acids) with a putative signal sequence of 25 amino acids (Table I). The mature polypeptide (304 amino acids, $M_r$ 32,947) is cationic (theoretical pI, 8.5).

Peroxidases with acidic or close to neutral pI values (anionic and moderately anionic), similar to TPX1, are secreted to the cell wall (Lagrimini et al., 1987; Roberts and Kolattukudy, 1989) and are thought to be involved in cell wall biosynthesis. Regarding TPX2, the function and localization of cationic peroxidases are unclear. From biochemical studies, it was known that basic tobacco peroxidases are localized in vacuoles (Johansson et al., 1992). Moreover, some cationic peroxidases are processed in the C terminus, which may imply that they are sorted to vacuoles (Johansson et al., 1992). However, the major isoform present in the extracellular medium of peanut suspension cells is a cationic peroxidase (Buffard et al., 1990). This is a clear indication that not all cationic peroxidases are targeted to vacuoles. When we align the C terminus of TPX1 and TPX2 with peroxidases reported to be secreted to the cell wall, including the peanut cationic peroxidase (Buffard et al., 1990) and tobacco and tomato anionic peroxidases (Espelie et al., 1986; Lagrimini et al., 1987), all of them lack the C-terminal extension that is present in predicted vacuolar peroxidases. This indicates that both TPX1 and TPX2 may be targeted to the cell wall.

**Table I.** *Characteristics of two peroxidase genes from tomato*

| |
|---|
| Organism: |
| *Lycopersicon esculentum* L. cv Pera. |
| Location on Chromosome: |
| Not known. |
| Function: |
| Peroxidases (donor:hydrogen-peroxide oxidoreductase, EC 1.11.1.7). |
| Source: |
| λgt10 cDNA library constructed from mRNA isolated from 15-d-old plants. |
| Techniques: |
| Screening cDNA library using a degenerate oligonucleotide corresponding to the acid-base catalytic region. |
| Method of Identification: |
| Nucleotide and deduced amino acid sequence comparison with conserved regions of known peroxidases. |
| Expression Characteristics: |
| Both clones were expressed in roots and hybridize with transcripts of approximately 1300 bases. |
| Features of Amino Acid Sequences: |
| TPX1: 328 amino acids; a putative 22-amino acid signal peptide, based on the mature protein sizes and homologies with other peroxidases. TPX2: 329-amino acid mature protein with a putative signal peptide of 25 amino acids. |
| Subcellular Localization: |
| Not tested, but the presence of putative signal peptide sequences suggests that transmembrane transport may occur. |

[1] M.A.B. was supported by a fellowship from the Direccion General de Investigacion Cientifica y Tecnica (DGICYT), Spain. Some financial support was obtained from DGICYT, grant No. AGR91-0858-C02-01.

* Corresponding author; fax 34–52–132000.

Abbreviation: pI, isoelectric point.

## ACKNOWLEDGMENTS

The excellent technical assistance of Ms. Jean Clithero and the assistance of Ms. Becky Fagan and Mr. Jian-Kang Zhu in the preparation of this paper is gratefully acknowledged.

Received April 23, 1993; accepted May 18, 1993.



The EMBL accession numbers for the sequences of TPX1 and TPX2 are L13653 and L13654, respectively.

## LITERATURE CITED

**Abeles FB, Biles CL** (1991) Characterization of peroxidases in lignifying peach fruit endocarp. Plant Physiol **95:** 269–273

**Buffard D, Breda C, van Huyste RB, Asemota O, Pierre M, Daug Ha DB, Esnault R** (1990) Molecular cloning of complementary DNAs encoding two cationic peroxidases from cultivated peanut cells. Proc Natl Acad Sci USA **87:** 8874–8878

**Espelie KE, Franceschi VR, Kolattukudy PE** (1986) Immunocytochemical localization and time course of appearance of an anionic peroxidase associated with suberization in wound-healing potato tuber tissue. Plant Physiol **81:** 487–492

**Gaspar T, Penel C, Hagege D, Greppin H** (1989) Peroxidases in plant growth, differentiation, and developmental processes. *In* J Lobarzewski, H Greppin, C Penel, T Gaspar, eds, Biochemical, Molecular, and Physiological Aspects of Plant Peroxidases. University M Curie-Sklodowska Lublin, Poland, and University of Geneva, Switzerland, pp 249–280

**Johansson A, Rassmusen SK, Harthill JE, Welinder KG** (1992) cDNA, amino acid and carbohydrate sequence of barley seed-specific peroxidase BP 1. Plant Mol Biol **18:** 1151–1161

**Lagrimini LM** (1991) Wound-induced deposition of polyphenols in transgenic plants overexpressing peroxidase. Plant Physiol **96:** 577–583

**Lagrimini LM, Burkhart W, Moyer M, Rothstein S** (1987) Molecular cloning of complementary DNA encoding the lignin-forming peroxidase from tobacco: molecular analysis and tissue specific expression. Proc Natl Acad Sci USA **84:** 7542–7546

**Roberts E, Kolattukudy PE** (1989) Molecular cloning, nucleotide sequence and abscisic acid induction of a suberition-associated highly anionic peroxidase. Mol Gen Genet **217:** 223–232

**Welinder KG** (1985) Plant peroxidases: their primary, secondary and tertiary structures, and relation with cytocrome c peroxidase. Eur J Biochem **151:** 497–504

**Ye XS, Pan SQ, Kuc J** (1990) Activity, isoenzyme pattern, and cellular localization of peroxidase as related to systemic resistance of tobacco to blue mold (*Perospora tabacina*) and to tobacco mosaic virus. Phytopathology **80:** 1295–1298